

**COVER**

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**TITLE:** X-ray Crystallography of Botulinum Neurotoxins

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**CONTRACTING ORGANIZATION:** Department of Veterans Affairs Medical Center  
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# REPORT DOCUMENTATION PAGE

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13. ABSTRACT (Maximum 200 words)  Botulinum Neurotoxin type E was purchased, and the sample was crystallized although under slightly different conditions than we previously used. Thin plate-like crystals were obtained. Cryogenic conditions have been established and we were able to freeze the crystals for data collection. Native x-ray data sets have been collected to approximately 3.2 Angstrom resolution.					
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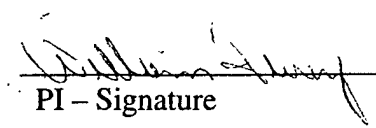
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PI - Signature

10/1/99  
Date

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11. Comments on administrative and logistical matters.

There was a delayed start of the project, caused by confusion over notification of the actual starting date, and by a loss of several technical people named in the original proposal during the period between proposal submission and our being made aware of the funding decision. Once the funding decision was made known to us, it took roughly eight months to recruit suitable new postdocs to replace those who left. As a consequence the project did not actually begin until March 1, 1999, and appropriately no funds were requested until February 12, 1999. The first fund transfer was received by the VA on February 23, 1999 to cover the upcoming initial three month period. We did however, purchase some of the protein toxin with VA funds ahead of time so that work could proceed immediately upon arrival of the new personnel. Assuming March 1, 1999 as the starting date for the project, this document covers the first and second quarterly progress reports. Correspondence with Judy Pawlus informed us however, that the first ANNUAL report is due; although we don't really understand why as apparently there is confusion over the actual starting date. Note that even now, we still have not received a copy of the contract or statement of work. In her correspondence Judy stated that this report could serve as both the quarterly reports and annual report provided it includes certain information, which it now does. A copy of our early correspondence with Judy and her response is included in the appendix. Based on subsequent conversations and the fact that several key administrative people were on vacation, it was decided that perhaps an additional delay would be in order until the starting date issue is resolved. I have recently spoken to Major Charles Millard (the newly appointed contracting officer) about this and requested that the TERMINATION date be amended such that the original project duration is maintained, but the starting date reflect that for which we actually started the work (March 1, 1999). This would require a change in the termination date from 9/30/01 to 2/28/03. There would be essentially no additional costs associated with this change. Major Millard is looking into this but hasn't got back to me yet.

12. Describe scientific progress for the period in terms of the tasks or objectives listed in the statement of work for this contract. Explain deviations where this is not possible. Include available data. Use additional pages, if necessary.

a) Botulinum neurotoxin type E was purchased from the Food Research Institute, Wisconsin. For reasons not yet understood we had to change our crystallization conditions relative to those we previously used. Nevertheless, we did eventually obtain crystals that are very thin plates. We are currently trying microseeding techniques to obtain larger and thicker crystals.

b) With the thin crystals available we have successfully determined cryogenic conditions suitable to freeze the crystals for x-ray data collection.

c) Native data sets have been collected on the thin crystals to roughly 3.2 Angstrom resolution.

d) A search for heavy atom derivatives has been initiated.

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13. Provide a brief statement of plans or milestones for the next quarter.

a) We will try to improve the crystal size and quality by additional microseeding experiments and by searching for alternative crystallization conditions.

b) We plan to collect higher resolution diffraction data from native crystals.

c) We will try to use the molecular replacement (MR) method to solve the native BoNT/E structure using the recently solved BoNT/B crystal structure as a search model. However, we will collect at least one derivative data set and combine isomorphous replacement and MR phase information to determine the structure.

d) Our experience with BoNT/B showed that better quality crystals are obtained when the protein is complexed with inhibitors (mostly zinc chelating agents). We will try the same technique with the BoNT/E protein.

- b) Response received by e-mail from Judy Pawlus regarding the message in (a). This response was received June 9, 1999.

From: IN%"Judy.Pawlus@DET.AMEDD.ARMY.MIL" "Pawlus Judy K" 9-JUN-1999 13:15:19.37  
To: IN%"FUREYW@vms.cis.pitt.edu" "'FUREYW@vms.cis.pitt.edu'"  
CC:  
Subj: RE: MIPR 8827

Return-path: <Judy.Pawlus@DET.AMEDD.ARMY.MIL>  
Received: from dasmthefl803.amedd.army.mil ("port 1394"@[140.139.7.194])  
by vms.cis.pitt.edu (PMDF V5.1-10 #22606)  
with ESMTP id <01JC74MLCK009AXULS@vms.cis.pitt.edu> for  
FUREYW@vms.cis.pitt.edu; Wed, 9 Jun 1999 13:15:05 EDT  
Received: by AMEDD-DET.AMEDD.ARMY.MIL with Internet Mail Service (5.5.2232.9)  
id <J5JQX7RW>; Wed, 09 Jun 1999 13:15:00 -0400  
Content-return: allowed  
Date: Wed, 09 Jun 1999 13:14:50 -0400  
From: Pawlus Judy K <Judy.Pawlus@DET.AMEDD.ARMY.MIL>  
Subject: RE: MIPR 8827  
To: "'FUREYW@vms.cis.pitt.edu'" <FUREYW@vms.cis.pitt.edu>  
Message-id:  
<FB65994695BFD211B02A00A0C9EA3319321569@dasmthefl799.amedd.army.mil>  
X-Envelope-to: FUREYW  
MIME-version: 1.0  
X-Mailer: Internet Mail Service (5.5.2232.9)  
Content-type: text/plain; charset="ISO-8859-1"

It is the first annual report that is due as well. You indicate you are writing quarterly reports. We can use the quarterly report as your first annual only if it states the information below. You should report negative as well as positive findings. If problems were encountered this is the information one would report in their first annual. Use the cover, SF298 and Foreword page add Table of Contents and attached the Quarterly report as you indicated below. Forward to MCMR-RMI-S as instructed in our letter. This will satisfy request from RMIS as well as Joyce Maylone.

-----Original Message-----

From: FUREYW@vms.cis.pitt.edu [mailto:FUREYW@vms.cis.pitt.edu]  
Sent: Wednesday, June 09, 1999 1:02 PM  
To: judy.pawlus@det.amedd.army.mil  
Subject: MIPR 8827

Message as in (a).